

Amendments to the Claims:

Please amend the claims to read as follows:

1. (Currently Amended) A method for isolating compounds that possess amyloid inhibitory activity from plant matter of the genus *Uncaria*, the method comprising the steps:
  - a) preparing a polar solvent extract of *Uncaria* plant matter, where the polar solvent extraction is selected from one of the extraction methods from the group of extraction methods consisting of extraction with water, extraction with a water solution of a polar alcohol, extraction with a water solution of acetonitrile and extraction with a water solution of another polar organic solvent selected from the group of polar organic solvents consisting of triethanolamine, acetone, and the like, and running the extract through a first column that comprises hydroxy group containing resin, resin having hydrophobic characteristics but without any hydroxy groups, or a mixture of both;
  - b) eluting the first column with distilled water, followed by eluting with not more than 2-4 column bed volume washings with a dilute polar alcohol/water solution having an alcohol/water ratio not greater than 50/50, and discarding any eluate;
  - c) eluting the first column with one or more column bed volume washings of a polar alcohol/water solution having an alcohol/water ratio between 50/50 and substantially pure alcohol, and collecting and drying the eluted volumes to a dried material.
2. (Currently Amended) The method of claim 1 wherein the column that comprises hydroxy containing resin, resin having hydrophobic characteristics but without any hydroxy groups, or a mixture of both is a column selected from the group of columns consisting of ~~C2 column, C4 column, C18 column, and the like~~ carbon-containing columns, Tris-acrylate column, LH-20 column, and Affi-prep 10 gel column, and the like.
3. (Original) The method of claim 1 wherein the polar alcohol/water solution has an alcohol/water ratio of 75/25 or higher.
4. (Original) The method of claim 1 wherein the washing in step (c) is effected with substantially pure methanol.
5. (Original) The method of claim 1 wherein the plant matter of the genus *Uncaria* is taken

from one or more of the plants of the various *Uncaria* species plant group consisting of *tomentosa*, *attenuata*, *elliptica*, *guianensis*, *pteropoda*, *bernaysli*, *ferra* DC, *kawakamii*, *rhyncophylla*, *calophylla*, *gambir*, and *orientalis*.

6. (Original) The method of claim 1 wherein the plant matter of the genus *Uncaria* is taken from *Uncaria tomentosa*.

7. (Original) The method of claim 6 wherein the *Uncaria tomentosa* plant matter is taken from one or more of the group of plant parts consisting of inner bark and root.

8. (Original) The method of claim 1 further comprising the steps:

d) applying an aqueous solution of the dried material from step (c) to a second column comprising a hydrophobic resin, the second column having been preparatorily equilibrated in a solvent comprising about 95% water/5% acetonitrile, referred to herein as solvent A, and then eluting the second column with more solvent A and discarding the eluate;

e) eluting the second column with a mixture of solvent A containing 10-15% of a solvent comprising about 95% acetonitrile/5% water, referred to herein as solvent B, and collecting and drying the eluted volumes to a dried material.

9. (Currently Amended) The method of claim 8 wherein the second column comprising a hydrophobic resin is a column selected from the group of columns consisting of C18 SPE, a flash chromatography column, ~~Varian Chroma-Zone™~~, other HPLC columns, and other carbon-containing columns, ~~and the like~~.

10. (Currently Amended) The method of claim 1 or 8 further comprising the steps:

f) making one or more injections of a solution of the dried material of step (c) or the dried material of step (e) in a solvent selected from the group of solvents consisting of water, water/dilute alcohol and a solvent A comprising about 95% water/5% acetonitrile and no more than 10% of a solvent B, comprising about 95% acetonitrile/5% water, into an HPLC instrument having a diode array uv/vis detector with a graphic display, the HPLC instrument further comprising a reverse-phase column;

g) eluting the material through the HPLC column using a solvent gradient profile as follows: 10% solvent B for about the first 20 minutes from start of elution, 10 to 100%

solvent B gradient for about minutes 20 to 30 from start of elution, and 100 to 10% solvent B gradient for about minutes 30 to 32 from start of elution, while observing the uv/vis detector graphic display during the elution gradient over time, and separating fractions of the eluate at elution times corresponding to times associated with the graphic display peaks.

11. (Original) The method of claim 10, wherein the reverse-phase column has dimensions of about 2.2cm X 25cm and contain about 95ml of C18 reverse phase resin, wherein the solution of the dried material is a solution of about 50 mg of the dried material of step (c) in about 1-2 ml of solvent A, wherein the step of injecting the solution of dried material into the HPLC may be repeated, wherein a HPLC column solution gradient flow rate is set to about 5 mls per minute, and further wherein the solvent gradient profile is 10% solvent B for 0 to 20 minutes, followed by 10 to 100% solvent B gradient for minutes 20 to 30, and 100% to 10% solvent B gradient from minutes 30 to 31; such that fractions F though N of the eluate are collected at the following times: fraction G (13-14 minutes), fraction F (15-16 minutes), fraction H (17-20 minutes), fraction I (21 minutes), fraction J (22 - 23 minutes), fraction K1 (24 minutes), fraction K2 (25 minutes), fraction L (26-27 minutes), fraction M (27-28 minutes), and fraction N (28-29 minutes).

12. (Original) The method of claim 10, wherein the reverse-phase column with dimensions of 1.0 cm X 25.0 cm containing 20ml of C18 reverse phase resin, wherein the solution of the dried material of step (c) is a solution of 50 µg of the dried material in 50-100µl of solvent A, wherein the step of injecting the solution into the HPLC is repeated multiple times, wherein a HPLC column solution gradient flow rate is set to about 1.5 mls per minute, and further wherein the solvent gradient profile is 10% solvent B for 0 to 20 minutes, followed by 10 to 100% solvent B gradient for minutes 20 to 30, and 100% to 10% solvent B gradient from minutes 30 to 31; such that fractions F though O of the eluate are collected at the following times: fraction G (12-13 minutes), fraction F (13-14 minutes), fraction H (15 minutes), fraction I (16 minutes), fraction J (18-19 minutes), fraction K1 (20 minutes), fraction K2 (21 minutes), fraction L (21-23 minutes), fraction M (23 minutes), fraction N (24 minutes), and fraction O (26-27 minutes).

13. (Original) The method of claim 10 wherein steps (f) and (g) are as follows:

f) injecting a solution of 1 gram of the dried material of step (c) in 5 - 10 ml of solvent

A into an HPLC instrument having a Varian model 320 uv/vis detector set at 230 nm with a graphic display, the HPLC further comprising a 4.14 cm X 25 cm Varian Dynamax column further comprising 380 ml of C-18 reverse phase resin, the column fitted to a Varian Prostar 215 solvent delivery system, or the like.

g) eluting the HPLC column at a solution gradient flow rate of about 50 ml/minute, and further wherein the solvent gradient profile is with a solvent C/solvent D gradient as follows: 0-4 minutes, 25% D; 4-11 minutes, 25-30% D gradient; 11-14 minutes, 30-90% D gradient; 14-17 minutes, 90% D; and 17-19 minutes, 90-25% D gradient, where C is water and D is methanol, such that fractions F through O of the eluate are separated at elution times corresponding to times associated with the graphic display peaks.

14. (Original) The method of claim 1 wherein the preparation in step (a) of the extract of *Uncaria* is as follows:

- 1) adding 4000ml of methanol to 1 kg of *Uncaria tomentosa* and mixing
- 2) centrifuging the mixture at X2,500g using a centrifuge for 30 minutes and collecting the supernatant;
- 3) extracting the insoluble material about 3 more times as steps a and b above;
- 4) combining the supernatants and evaporating to a dried extract, or to at least about 500 ml volume, using a rotary evaporator at 50°C;
- 5) washing the dried extract, or the 500ml volume, 4 times with 300ml of petroleum ether, and discarding the ether layer;
- 6) further evaporating any remaining methanol to dryness using a rotary evaporator at 50°C;
- 7) extracting the dried extract 5 times with 150ml of distilled water, followed by centrifugation at 2,500Xg for 30 minutes each time, and
- 8) combining the supernatants and then lyophilizing using a freeze-dryer.

15. (Original) The method of claim 14 wherein the further preparation of the extract of *Uncaria* from the resulting lyophilized extract includes the following additional steps:

- 9) dissolving the resulting lyophilized extract into about 500 ml of distilled water, and applying 50-100ml portions to a 400 ml LH-20 column equilibrated with distilled water.

- 10) eluting the LH-20 column with 1,100ml of distilled water (~3 column volumes) and discarding the amber/yellow, non-active fractions;
- 11) eluting the LH-20 column with 1,100ml of 100% methanol (~3 column volumes) and collecting a set of active fractions and evaporating to dryness using a rotary evaporator at 50°C.
16. (Original) The method of claim 8 wherein the aqueous solution of a dried material from step (c) is further prepared by the following steps:
- 1) dissolving the dried material in water at 80 mg/ml and applying 5 ml at a time to a disposable C18 SPE column (10 gram) equilibrated in a first solvent comprising about 95% water/5% acetonitrile/ 0.1% TFA;
  - 2) washing with 3 column bed volumes of the first solvent and discarding the eluate.
  - 3) eluting with 3 column bed volumes of the first solvent further comprising about 12.5% of a second solvent comprising about 95% acetonitrile/5% water/0.1% TFA, and
  - 4) lyophilizing the corresponding fractions using a freeze-dryer.
17. (Currently Amended) The method of claim 8 wherein the aqueous solution of a dried material from step (c) is further prepared by the following steps:
- 1) dissolving the lyophilized fractions at 5 grams in 20 ml water and applying 20ml at a time to a ~~Varian Chroma..Zone™ apparatus~~ flash chromatography column,
  - 2) washing with 3 column bed volumes of a first solvent comprising about 95% water/5% acetonitrile/ 0.1% TFA and discarding the eluate;
  - 3) eluting with 3 column bed volumes of the first solvent further comprising about 12.5% of a second solvent comprising about 95% acetonitrile/5% water/0.1% TFA, and
  - 4) collecting and drying the next 3 column bed volumes of eluate.
- 18-21. (Cancelled)
22. (Original) The method of claim 1 further comprising the steps:
- d) applying an aqueous solution of the dried material from step (c) to a second column, LH-20 or the like, eluting the material from the column with successive column volumes of water/methanol mixtures containing 0.1% TFA, beginning with 25% methanol and

increasing to 100% menthol in 25% increments, and collecting and combining the fractions;

e) separating, combining and drying a fraction to a dried material, referred to hereafter as compound H, by analytical HPLC, the fraction containing a peak occurring between 7-8 minutes from start of elution on a Dynamax 5 $\mu$  C-18 column having dimensions of about 4.6mm X 25cm, using an elution gradient of water for solvent A and methanol for solvent B, A and B each containing about 0.1% TFA, with detection at 280 nm, the gradient conditions being 0 to 9 min for 25% to 36% B gradient, 3 to 10 min for 36 to 100% B gradient, 10 to 12 min for 100 % B and 12 to 13 min for 100 to 25% B gradient, all at a flow rate of about 20 ml/min;

f) making one or more injections of a solution of the dried material of step (e) above in a solvent comprising water/methanol 80/20 containing about 0.1% TFA and applied at about 150 mg/run to a preparative HPLC Dynamax 5 $\mu$  C-18 column with dimensions of about 21.4mm X 25cm, using substantially the same elution gradient as used in step (e) above, with detection at 280 and 300 nm, the gradient conditions being 0 to 3 min for 20% to 25% B gradient, 3 to 9 min for 25 to 45% B gradient, 9 to 10 min for 45 to 100% B gradient, 10 to 12 min for 100% B and 12 to 13 min for 100 to 25%B gradient, all at a flow rate of about 20 ml/min, the compound H fraction eluting between 7-8 minutes from start of elution, and ;

g) repeating steps (e) and (f) above until the peak as seen on analytical HPLC in step (e) is relatively pure.

23-38. (Cancelled)

39. (New) The method of claim 1 wherein the polar organic solvent of step (a) is selected from the group of polar organic solvents consisting of triethanolamine, and acetone.

40. (New) The method of claim 2 wherein the carbon-containing columns are selected from the group consisting of C2 column, C4 column and C18 column.